VITAMIN A DEFICIENCY AND NIGHT BLINDNESS

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One of the oldest diseases known to man is nutritional night blindness. Its descriptions go back to the ancient Egyptian medical papyri and are already accompanied by the correct prescription for its cure, the eating of liver. Toward the end of World War I the factor in liver which cures night blindness was identified with the then newly discovered vitamin A.[1]

Vitamin A is the precursor in the retina of the visual pigments of the rods and cones.[2] It seems reasonable to suppose that on a diet deficient in this factor the retina eventually synthesizes subnormal amounts of visual pigment, with the corresponding decline of visual sensitivity that constitutes night blindness.

Some of the first studies of experimental human night blindness seemed to reveal such a simple and direct relationship.[3] In two subjects deprived of vitamin A, the visual thresholds of both rods and cones began at once to rise, until a mild night blindness had been established.[4] On oral administration of vitamin A or carotene, the thresholds of both rod and cone vision returned to normal within 2–3 hours.

It looked for a time, therefore, as though this might be an exemplary instance of the origin and cure of a biochemical disease, all elements of which were well understood. Further studies, however, exposed two major discrepancies: (1) Though in some subjects placed on a vitamin A–deficient diet the visual threshold began at once to rise, in a larger number it remained unchanged for periods ranging from several months[5] to, in one instance, 2 years.[6] (2) Among the subjects who developed night blindness, some were completely cured within a few hours after receiving vitamin A, whereas others, though showing some immediate improvement, took months of vitamin A supplementation to return to normal.[7]

One might take a simple position with regard to the first of these discrepancies. The amounts of vitamin A stored in the livers of healthy human subjects are known to vary enormously.[8] In Britain, for example, Moore[6] found reserves in adults during 1941–44 ranging from about 7 to 750 μg/gm. If we take 1,500 gm. as the average weight of the adult liver and about 300 μg. (about 1,000 I.U.) as the daily drain upon stored vitamin A, the average Briton stores enough vitamin A in his liver—if used economically—to tide him over some 500 days of total deprival. An unusually well-supplied Briton—if we can disregard spoilage—might survive seven times as long, or almost 10 years! On the other hand, the most poorly supplied members of this group might have run through their stored vitamin A within 1–2 weeks. It is not difficult to understand, therefore, why most subjects taken from ordinary American or British environments fail to respond to vitamin A–deficient diets within months or even years. It is less clear why a fairly large proportion of them responded within a few days, even though in some instances highly supplemented with vitamin A for the preceding period.[3]

The second discrepancy—the great variability in the times required to cure night blindness—raises other issues. The visual pigments are composed of vitamin A

aldehyde (retinene) joined to specific proteins of the rods and cones called "opsins." The amounts of visual pigment that can be formed in the normal retina are limited, not by vitamin A, which is ordinarily present in excess, but by opsin. In thinking about night blindness, we have tended in the past to be too much preoccupied with vitamin A and have paid too little attention to the opsins.[2]

When one does consider the opsins, this at once suggests further relationships. The outer segment of a rod—and this must be true also of many cones—is composed in considerable part of visual pigment, that is, of opsin, since the retinene chromophore constitutes only about 1 per cent of these molecules. Opsin accounts for about 40 per cent of the dry weight of the outer segment of a frog rod and 14 per cent of that of a cattle rod.[2] It is an important structural constituent of the rods and probably of the cones; and any loss of this protein might be equivalent to the structural deterioration of the visual receptors.

Tansley[9] showed some years ago that in vitamin A–deficient rats and dogs, somewhat later than the decline in rhodopsin production that should have initiated night blindness, the outer segments of the rods deteriorated structurally. Johnson[10] confirmed and extended these observations in the rat; and recently similar changes have been observed in both rods and cones of the monkey.[11] According to Johnson, after 7–13 weeks of vitamin A deprivation in young rats, many outer segments have disappeared, and those that remain stain abnormally. As the deficiency progresses, the inner segments of the rods also degenerate, and then successively the external limiting membrane, the outer nuclear layer, and the inner nuclear layer. These changes occur sooner in central than in peripheral areas of the retina. The outer segments of rods which have deteriorated only slightly seem to repair considerably within 24 hours of feeding vitamin A. Even rods which have degenerated completely seem to be replaced within 10–18 weeks of vitamin A supplementation.

These observations suggest that the time required to cure night blindness may depend on the extent to which vitamin A deficiency has altered the retinal structure. Simple lack of vitamin A, through lowering the concentrations of visual pigments, might induce a night blindness that is cured as rapidly as vitamin A re-enters the retina; but the structural deterioration of the retina, heralded perhaps by the loss of opsin, might take much longer to repair.

For these reasons it seemed worthwhile to map the entire course of vitamin A deficiency and its cure in the rat. In single groups of animals we have measured simultaneously the vitamin A in the liver and blood, the retinal content of rhodopsin and opsin, the electroretinographic threshold, and the ERG's obtained over a wide range of light intensities. In key instances we have also examined the retinal histology.

Not all these things were done for the first time. We have already discussed the histological studies of Tansley and Johnson and should mention particularly also Tansley's fine study of rhodopsin synthesis in normal and vitamin A–deficient rats[12] and the measurements of liver, blood, and retinal vitamin A in normal and deficient rats by Lewis, Bodansky, Falk, and McGuire.[13]

Plan of the Experiments.—A number of experiments were performed, all of which yielded substantially the same pattern of results. We shall describe primarily the last such experiment, because it brings together all the procedures and represents most completely and typically all our observations.

Male albino rats of the highly inbred Harvard colony, 22–24 days old and weighing 36-66 gm., were divided into two groups, one kept on the complete laboratory ration, the other placed on the standard USP vitamin A test diet. The animals on the deficient diet continued to gain weight for about 5 weeks, though more slowly than normal. At this time they weighed an average of 112 gm. as compared with the control weight of 215 gm. but were altogether normal in appearance. In the fifth to seventh weeks their weights plateaued and thereafter declined rapidly. At the same time—in the seventh and eighth weeks— the classic overt signs of vitamin A deficiency appeared, and by the end of the eighth week all the animals not sacrificed in the experiments had died.

For electroretinography, animals that had been dark-adapted overnight were anesthetized with nembutal. The eye was held open with threads drawn through the lids. Cotton-wick electrodes were used, moistened with Ringer solution, one touching the side of the cornea, the other a shaved area on the cheek. The response was recorded with a capacity-coupled Grass P4 preamplifier and a Dumont oscilloscope with camera attachment. The stimuli were $1/50$-second flashes of white light, the intensity of which was controlled with neutral filters and photographic wedges. The absolute threshold was measured by starting with the light well below threshold and flashing it every few seconds at gradually increasing intensities until a response could be detected on the oscilloscope. This procedure was repeated until constant readings were obtained. Then the ERG was recorded over a wide range of intensities.

After dark-adapting overnight, the same animals were used next morning for the biochemical measurements. They were again anesthetized, the body cavity was opened, and 5–10 ml. of blood were taken from the heart with an oxalated syringe. The entire liver was removed and also both eyes. One eye of each animal was used to measure rhodopsin, the other to measure opsin; but, since each of these determinations requires 2 retinas, animals were paired, usually on the basis of having yielded comparable electroretinograms.

To determine blood vitamin A, the oxalated blood from one animal was centrifuged, and the clear plasma was mixed with an equal volume of ethyl alcohol and extracted three times with petroleum ether. This extract was transferred to 0.3 ml. of chloroform, and a micro-antimony chloride test was performed by mixing 0.25 ml. of the extract with 0.50 ml. of antimony chloride reagent, recording the absorption spectrum at once in a Cary recording spectrophotometer.

The livers, weighing 4–12 gm., were ground with anhydrous sodium sulfate to a fine powder and extracted by shaking with diethyl ether. An aliquot of this extract was transferred to chloroform, and its vitamin A content determined by the antimony chloride procedure.

To measure rhodopsin, two retinas were hardened in 4 per cent alum solution for 15–20 minutes, then washed with distilled water and buffer, and extracted overnight with 0.2 ml. of 2 per cent digitonin solution. After centrifuging, 0.01 ml. of 1 M hydroxylamine was added to the extract, and the absorption spectra recorded before and after bleaching. The change in extinction at 500 mμ measured rhodopsin.

Opsin was determined by measuring the capacity of retinas to regenerate rhodopsin when incubated with neo-b (11-*cis*) retinene.[14] We found that rat rhodopsin

in digitonin solution regenerates very little when bleached and incubated with neo-*b* retinene. For this reason whole retinas were exposed to bright light until wholly bleached. Then a large excess of neo-*b* retinene, dissolved in 0.025 ml. acetone, was added to the retinas suspended in buffer solution, the mixture was stirred periodically during 4–6 hours at room temperature and then left at 5° C. overnight. The rhodopsin which had formed was extracted and measured as described above. Control measurements showed that 70–80 per cent of rhodopsin originally present in a retina was regenerated and recovered by this procedure.
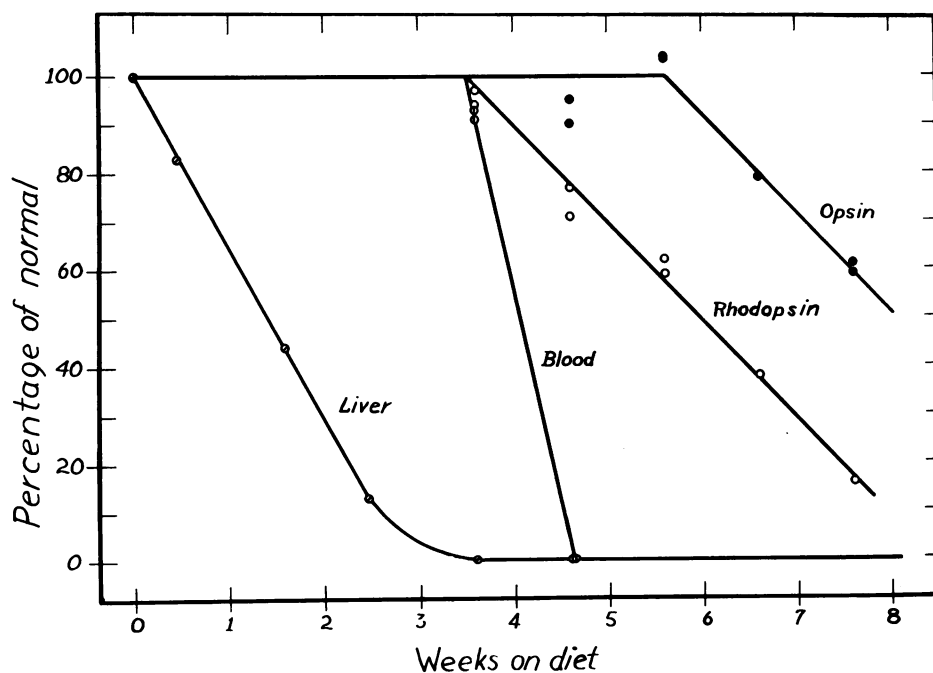

FIG. 1.—Biochemical changes in a group of white rats on a vitamin A–deficient diet. The animals were 22–24 days old when the diet was begun. The liver vitamin A began to fall at once and within 3 weeks had reached low values. Then within a week the blood level fell from normal to zero. With this, the rhodopsin content of the retina declined, marking the onset of night blindness. Later the opsin also declined, marking the beginning of the histological deterioration of the retina.

Vitamin A of Liver and Blood; Rhodopsin and Opsin.—Figure 1 shows in one group of animals the effects of the deficient diet on the vitamin A content of the liver, the vitamin A concentration in the blood, and the rhodopsin and opsin of the retina. The values are expressed as percentages of normal. For the liver this means the percentage remaining of the vitamin A present in control animals at the time the diet was begun. The blood vitamin A, rhodopsin, and opsin are expressed as percentages of the values found in control animals of the same age.

The liver vitamin A begins to fall as soon as the diet is begun and within 3 weeks has reached a very low value. This depletion proceeded at the average rate of 2–2.5 μg. daily, the withdrawal rate for animals of this age and weight. Meanwhile, the control animals on the complete diet increased their liver stores at an average rate of 45 μg. daily. It is this that makes the age at which the diet is

begun decisive for the course of the deficiency.   Our control animals when 53 days old had livers weighing, on the average, 16 gm. and containing 1,360 μg. vitamin A; withdrawn at a daily rate of even 5 μg., this might have tided them over 9 months of a deficient diet.

The blood maintains its normal concentration of vitamin A (10.4 μg. per cent in the deprived animals, 11.2 μg. per cent in the controls) until the liver has been

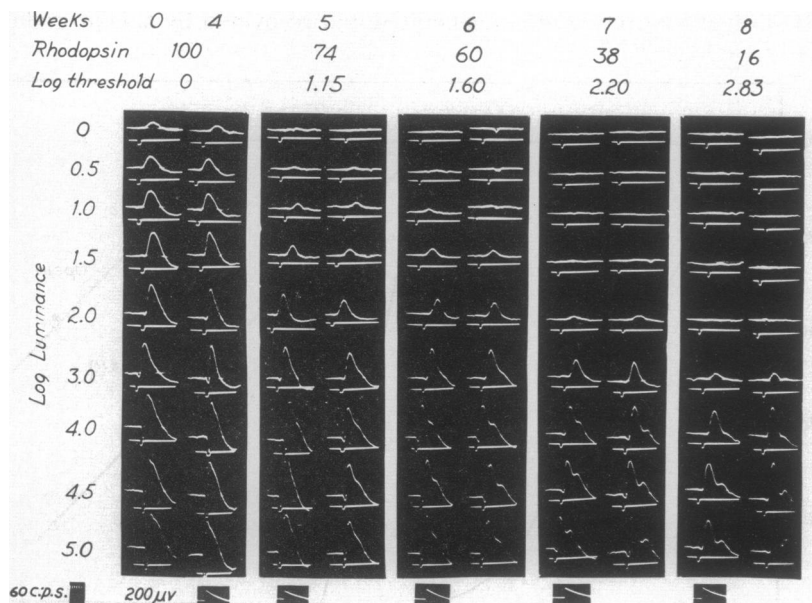| Weeks	0	4	5	6	7	8
Rhodopsin	100		74	60	38	16
Log threshold	0		1.15	1.60	2.20	2.83

Fig. 2.—Effects of vitamin A deficiency on the electroretinogram (ERG). The top three lines show the number of weeks on the deficient diet, the rhodopsin content of the retinas as percentages of normal; and the logarithm of the luminance of light needed to evoke a just perceptible ERG, the average threshold of normal animals being set arbitrarily at 1 (log threshold = 0).   ERG's are shown in response to a range of luminances of 5 log units, i.e., 1 to 100,000. Below each ERG, a marker shows the $^1/_{50}$-second flash.   The small rectangles at the bottom show a trace of the 60-cycle A.C. to indicate the time scale, and weekly calibrations of the oscillographic response to a pulse of 200 microvolts. The first two vertical rows of ERG's show responses from a rat about to begin the diet and those from another after 4 weeks, when the vision is still normal. Thereafter, records are shown from a pair of animals each week.   As the rhodopsin declines, the ERG threshold rises (night blindness), and the ERG displays characteristic changes: (*a*) the *b*-wave at each level of luminance declines; (*b*) the *a*-wave declines still more rapidly; and (*c*) an inflection on the downward sweep of the *b*-wave is delayed longer and longer until it appears as a separate positive wave.

emptied.   Then in the space of a few days the blood vitamin A falls precipitately to zero.

Up to this time the rhodopsin content of the retina remains normal.   The extract of two retinas in 0.21-ml. solution possesses an extinction at 500 mμ of 0.280, corresponding to a rhodopsin content of 7.24×10^{-4} μmoles per retina.   This is equivalent to a vitamin A content of 0.21 μg. per retina.   (Lewis *et al.*[13] found only one-fifth to one-third as much vitamin A in the rat retina; the description of their preparative procedure suggests that it may have involved large losses of rod outer segments.)

Now the rhodopsin also begins to fall and within 3 weeks has reached very low values.   As we shall see, this marks the beginning of night blindness.

There is a curious interval of 2–3 weeks in which, though the rhodopsin content has declined, the opsin level is still normal.   That is, with the liver and blood emptied of vitamin A, the retina contains opsin which can find no vitamin A with which to combine.

Then the opsin level, too, begins to fall, and this marks the beginning of the structural deterioration of the retina (see below).   At this time also—the seventh and eight weeks of the diet—the classic signs of vitamin A deficiency appear. (In another of our experiments it took 10–11 weeks to reach this stage.)   The
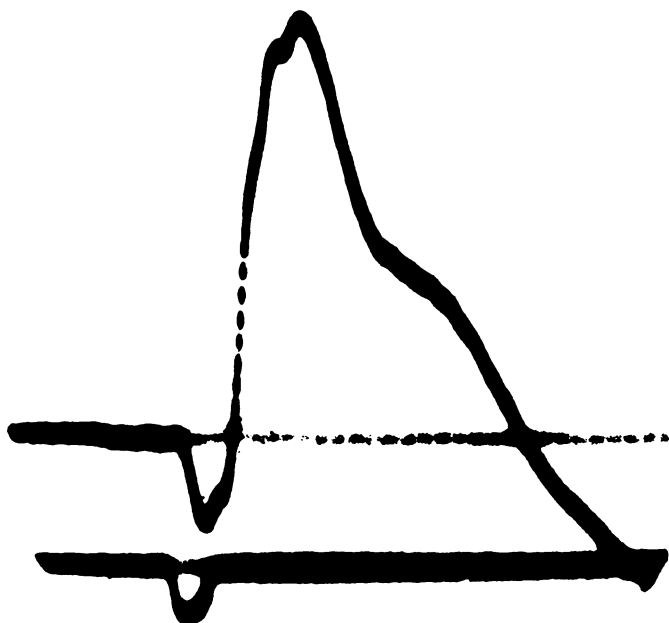

FIG. 3.—Form of the ERG.   Both the *a*- and *b*-waves are two-cusped.   This feature appears also in the human ERG, where the shorter-latency component in each wave is believed due to cone responses, the longer-latency component to rods.   The strong inflection evident on the downward sweep of the *b*-wave is delayed longer and longer as night blindness develops.   Below the ERG, a marker shows the $1/50$-second stimulating flash.

animals lose weight rapidly; and by the end of the eighth week all not already used in the experiments have died.[15]

Physiological Changes.—For the first 4 weeks on the diet, the animals appear to be physiologically normal.   During this time, first the liver and then the blood is depleted of vitamin A.   In the fifth week, as the rhodopsin level begins to fall, the visual threshold rises, marking the beginning of night blindness.

Figure 2 shows electroretinograms recorded in pairs of experimental animals. At the top of the figure are shown the number of weeks on the diet, and below this the average rhodopsin content of the retinas as percentages of the normal value. Below this is the logarithm of the threshold for a just perceptible retinogram; the normal threshold has been set arbitrarily at 1 (log threshold = 0), so that these

numbers represent the rise in log threshold over the normal value. The rat's eyes were exposed to $^1/_{50}$-second flashes at the log luminances shown on an arbitrary scale at the left. The figure shows a series of ERG's at various luminances for each of two rats each week. Since no changes occur in the first 4 weeks, the first pair of records involves one rat just about to begin the diet and another rat that had been 4 weeks on the diet.

At the end of the fourth week the ERG is entirely normal. In the fifth week, the rhodopsin level falls to 74 per cent, and the visual threshold rises 1.15 log unit, or about 14 times. In the succeeding weeks, as the rhodopsin level continues to
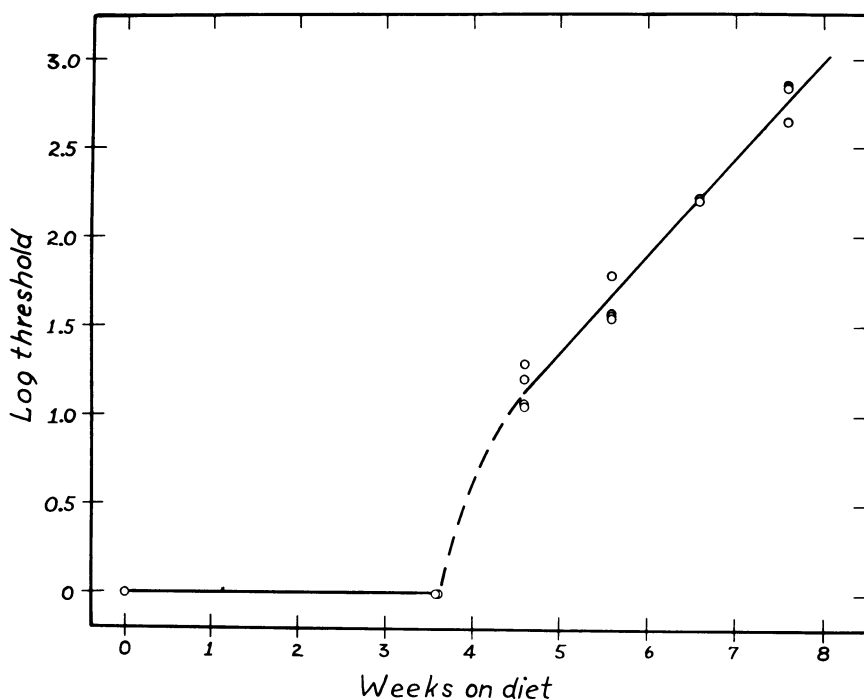

FIG. 4.—Development of night blindness with time on the vitamin A–deficient diet. Each point shows the logarithm of the ERG threshold of a single animal, the normal threshold being set arbitrarily at 1 (log threshold = 0). The log threshold rises linearly with time on the diet, following a more rapid initial rise.

decline, the threshold rises until, at the end of the eighth week, the rhodopsin is at 16 per cent, and the visual threshold has risen about 680 times.

Simultaneously, the ERG undergoes characteristic changes: (1) At all luminances but particularly at the lower ones, the height of the positive b-wave declines as the deficiency progresses. So, for example, at log luminance 3.0 the b-wave, nearly maximal at the end of the fourth week, has sunk to the just perceptible threshold level by the end of the eighth week. (2) The negative a-wave declines in amplitude still more rapidly. It is initially nearly as large as the b-wave at log luminance 5; but by the end of the eighth week it can hardly be elicited at all, even at this highest luminance. (3) A small positive inflection, which appears initially only as a hump on the downward sweep of the b-wave, is delayed longer and longer as the

deficiency progresses, until finally it has become a well-separated second positive wave, particularly evident at the higher luminances. The source of this delayed positive wave has not yet been identified. The possibility that it is an off-effect is not supported by our tests.

It may be noted in passing that some of the ERG's show clearly that both the a- and the b-waves are two-cusped. This is particularly evident in such records as those of the sixth week at log luminance 5. One of this pair of ERG's has been enlarged in Figure 3. Such two-cusped a- and b-waves have come to be associated in the human retinogram with the responses of cones and rods, the shorter latency component in each wave presumably representing the cone response.[16]
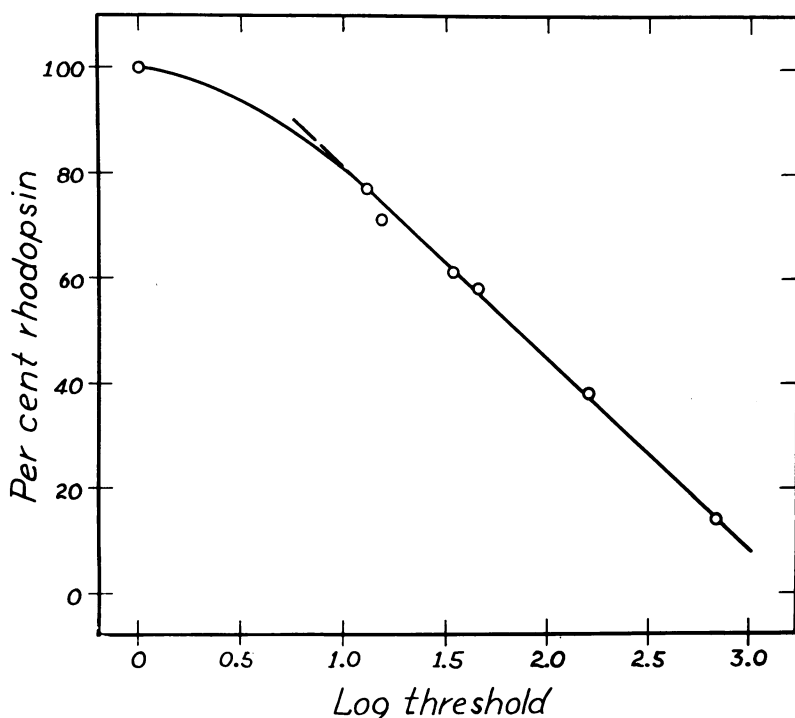

FIG. 5.—Rise of the ERG threshold with decline in rhodopsin content in the retinas of vitamin A–deficient rats. The normal log threshold is set at 0. Rhodopsin is expressed as the percentage of that found in control animals of the same age. Over most of its course the log threshold rises linearly as rhodopsin falls, following a disproportionately large rise of threshold with the first decline of rhodopsin.

It has frequently been asserted that rats possess only rods; yet Tansley[15] and Walls[17] state unequivocally that cones are present, and Sidman[18] has recently reported finding them in the approximate proportion 1 cone:10 rods. Since in the whole human retina the proportion of cones to rods is about 1:20, there may be as good anatomical basis in the rat as in man, for the two-cusped ERG's to represent cone and rod responses.

Figure 4 shows the relation between log threshold, and time on the deficient diet. After a somewhat abrupt start in the fifth week, the log threshold rises linearly. Similar behavior has been reported in man, the log threshold, rod and cone, rising linearly or nearly linearly for long periods on vitamin A–deficient diets.[3, 7]

Figure 5 shows the relation between the rhodopsin level of the retina and the logarithm of the visual threshold. Most of this relation again is linear, though distorted, as was Figure 4, by a somewhat disproportionate rise of threshold accompanying the first decline of rhodopsin. When the rhodopsin has fallen to half its normal content as the result of the deficiency, the threshold has risen about 100 times; when the rhodopsin is at 10 per cent, the threshold is up about 1,000 times. It would be interesting to determine whether in normal rats the bleaching of rhodopsin that accompanies light adaptation causes similar changes of threshold. It is now recognized that in the human eye the bleaching of a small fraction of rhodopsin raises the threshold enormously;[19] by one estimate, the bleaching of 0.6 per cent of the rhodopsin raises the threshold about 3,300 times.[20]

Histologic Changes.—The deterioration of retinal tissues in vitamin A–deficient rats has been described by Tansley[9] and by Johnson.[10] Our principal problem is
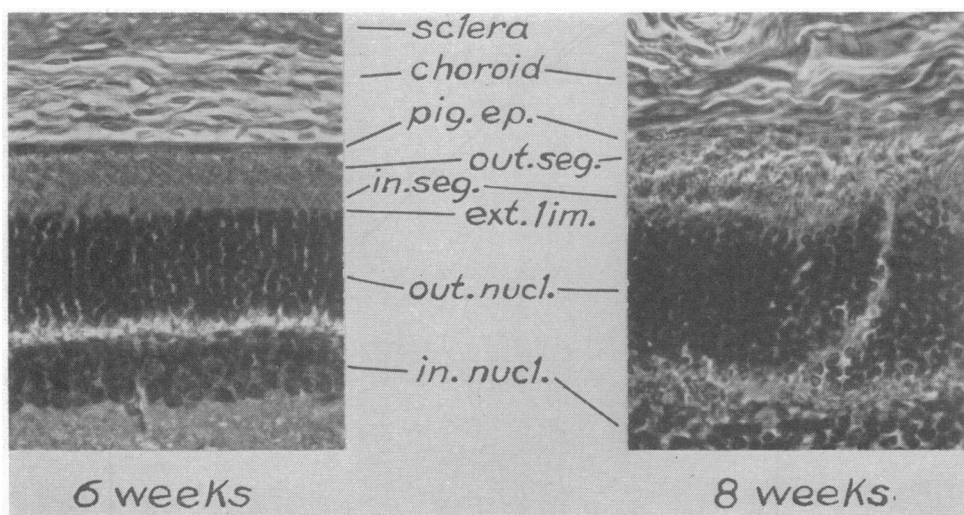

Fig. 6.—Effect of vitamin A deficiency on the structure of the rat retina. After 6 weeks on a vitamin A–deficient diet, the retina appears entirely normal. After 8 weeks it has deteriorated markedly: only traces remain of the pigment epithelium; the layer of rods, particularly the outer segments, is disintegrating; and the external limiting membrane has disappeared.

to orient these changes in the pattern of biochemical and physiological events that we have described.

After 6 weeks on the vitamin A–deficient diet, when the rhodopsin had fallen to about half its normal value but the opsin was still intact, the retinal histology of these animals appeared entirely normal (Fig. 6, *left*). After 8 weeks on the diet, however, when the opsin also had fallen to about half its normal value, the retinal tissues had deteriorated markedly (Fig. 6, *right*). The outer segments of the rods were attenuated, many had a gnawed appearance, and they were irregularly spaced. Only vestiges of the pigment epithelium remained. The sharp boundary that marked the external limiting membrane was gone; and the blood vessels of the choroid layer were frequently occluded.

Our supposition that, when opsin goes, the outer segments of the rods should deteriorate structurally has proved to be correct. By this time, however, the

animal is deteriorating generally. Not only are other retinal tissues affected as just described, but the superficial structures of the eye now begin to display the classic signs of vitamin A deficiency: corneal clouding, xerophthalmia, and secretion of a sticky red exudate about the eyes. The animal is losing weight rapidly, the coat is disarranged, some animals have developed an unsteady gait, some breathe with difficulty.

There is no compelling reason, therefore, to single out opsin among the animals' disabilities. To the degree that the loss of opsin is responsible for the histological decay of the outer segments of the rods, it may be only one of many proteins respon-
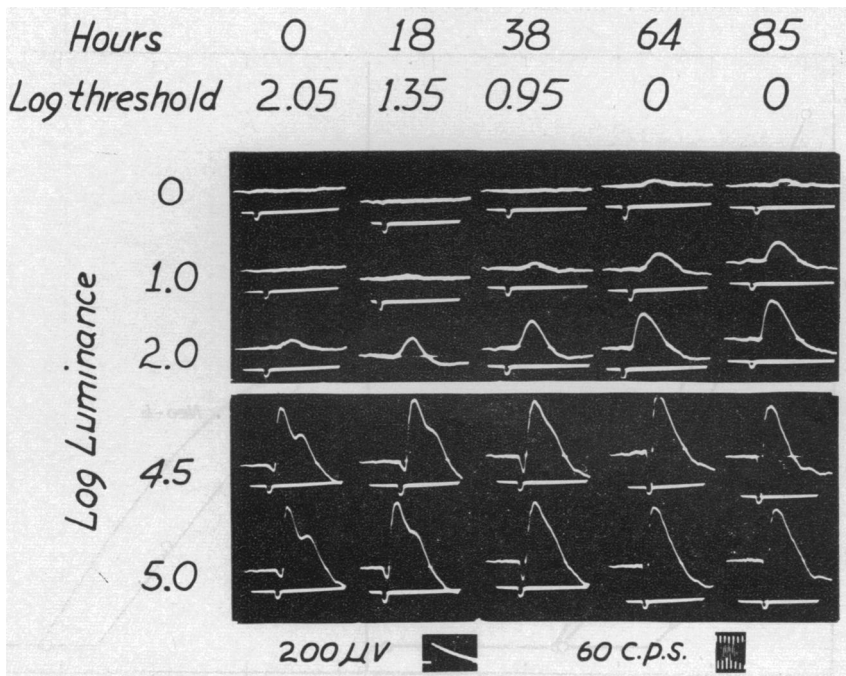

FIG. 7.—Recovery from night blindness on administration of vitamin A. Following intraperitoneal injection of a large dose of vitamin A, the ERG threshold returns within 64 hours to normal (log threshold = 0). During this interval the ERG retraces in reverse all the changes which had accompanied the development of night blindness.

sible for similar manifestations in many tissues. We shall have more to say of this below.

Recovery from Night Blindness.—A number of animals which have developed night blindness were "cured" by administering vitamin A. It was found that the vitamin could be supplied more effectively by intraperitoneal injection than orally. Oral administration yielded irregular results, successful in some deficient animals, not in others. It seemed as though some deficient animals had lost temporarily the capacity to take up vitamin A fed by mouth.

The effect of administering vitamin A to a night-blind animal is shown in Figure 7. At the beginning of the experiment this animal exhibited much the same ERG responses as did the animals of Figure 2 in the seventh week of the deficiency. Its ERG threshold was a little more than 100 times normal.

This animal was given a large dose of vitamin A—340 μg. dissolved in 1 ml. cottonseed oil, injected intraperitoneally. The ERG threshold slowly fell and within 64 hours had reached the normal level. Simultaneously, the ERG retraced in reverse all the changes that had accompanied the development of night blindness: the a- and b-waves increased to their former sizes, and the delayed positive wave was reincorporated into the b-wave.

Figure 8 shows the return of the ERG threshold to normal in a series of such recovery experiments. On the left are shown data from three deficient animals exhibiting various degrees of night blindness. Various amounts of vitamin A in cottonseed oil, ranging from 320 to 920 μg., were injected in 1–3 doses. In every
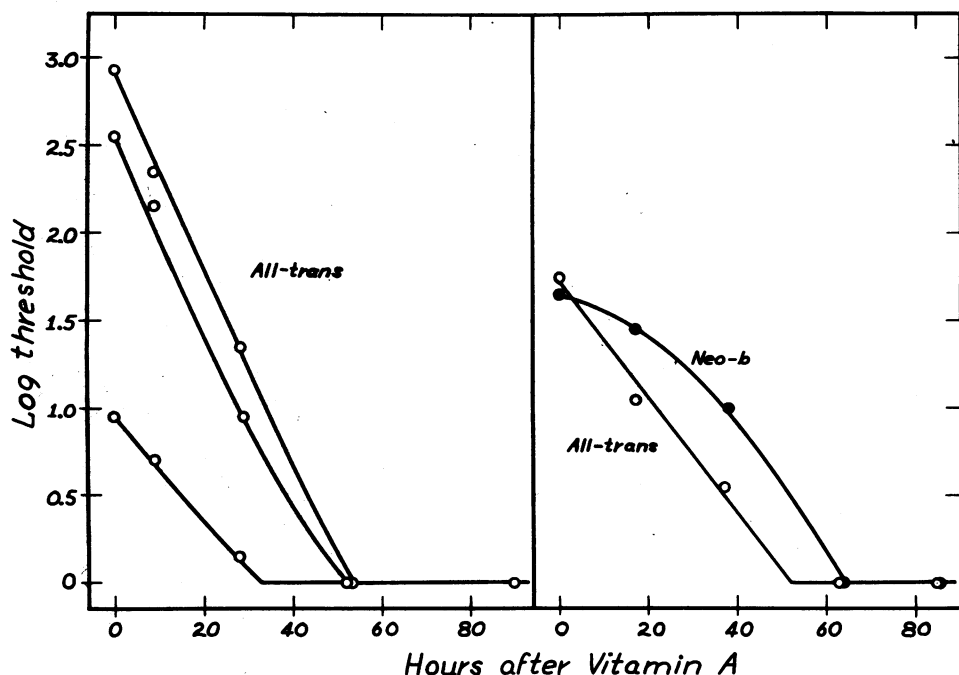

FIG. 8.—Recoveries from night blindness on administration of vitamin A. Log ERG thresholds measured at various times after intraperitoneal injection of large doses of all-*trans* vitamin A (*open circles*), or neo-b (11-*cis*) vitamin A (*closed circles*). After injection of the all-*trans* isomer, the log threshold falls almost linearly to the normal level; but with neo-b vitamin A there is a lag in recovery, caused apparently by the necessity to isomerize this configuration primarily to all-*trans* before it is used by the eye.

case the fall of log threshold to the normal level was approximately linear. The time for complete recovery depended primarily on the degree of night blindness, not the dosage level, at least within this range of high dosage.

In one instance two rats, closely matched in their degree of night blindness, were injected with equal amounts (*ca.* 1,000 μg.) of two geometric isomers of vitamin A: the all-*trans* form, which is most prevalent and which has been shown to be most effective in stimulating growth and liver storage in the rat,[21] and the hindered *cis* neo-b isomer (11-*cis*), which serves as precursor of the visual pigments. The result is shown at the right in Figure 8. The fall of log threshold on injection of all-*trans* vitamin A was, as usual, approximately linear; the neo-b isomer, however,

exhibited a distinct initial lag in its effect, though after 30–40 hours the responses to both isomers ran approximately parallel.

The vitamin A of the liver and blood was measured in these animals and in another pair which had been treated similarly. The animals injected with all-*trans* vitamin A had stored 71–87 μg. in the liver, in each case about 9 per cent of that injected, whereas those that received neo-*b* vitamin A had stored 32–39 μg., or about 4 per cent of that injected. Isomerization experiments conducted in both instances showed that none of the stored vitamin A was of the neo-*b* configuration. The blood levels after injection of either all-*trans* or neo-*b* vitamin A were normal (11.9 and 13.8 μg. per cent, respectively); again none of this was neo-*b*. Rhodopsin also was extracted from the retinas of the animals which yielded the data of Figure 8 (*right*). The extinctions at 500 mμ were 0.259 and 0.252, somewhat low, yet within the normal range.

It appears, therefore, that neo-*b* vitamin A, when injected intraperitoneally, is converted to other isomers, primarily all-*trans*, before entering the eye. It is presumably this process of isomerization that causes the observed delay in its action and may be responsible also for its relatively low effectiveness in growth and storage.[21]

Where this isomerization occurs is not yet known; but it appears to be a general feature of vitamin A metabolism, for surveys in our laboratory of representative tissues in fishes, rats, and cattle have failed to find the neo-*b* isomer anywhere but in the eye.[22] It seems likely that only other isomers of vitamin A are carried in the blood or stored in the liver and that the eye tissues themselves isomerize one or several of these to the neo-*b* configuration for the synthesis of visual pigments. The bleaching of the visual pigments by light, on the other hand, yields all-*trans* retinene and vitamin A; so that the visual processes include a continuous cycle of geometric isomerization between these two configurations.[14]

At the left of Figure 8 two recoveries are shown from states of night blindness involving 2.5–3 log units rise of threshold above normal. Figure 5 shows that this degree of night blindness corresponds to the decline of rhodopsin to 10–20 per cent of normal and Figure 1 that, by this time, opsin should have fallen to about 60 per cent of normal, with a corresponding disturbance of retinal histology. We cannot be certain that all these lesions occurred in the animals that yielded the data of Figure 8, but they were to be expected. The return of the threshold of these animals to normal 50 hours after administration of vitamin A implies that, within this interval, not only had neo-*b* vitamin A again been made available but that the retina had regained whatever opsin it had lost and had repaired whatever tissue deterioration had occurred. It should be said at once that these animals had been selected because their corneas were clear, since we wished to avoid the special complications of threshold measurement with cloudy corneas. The animals were, however, losing weight, and some of them exhibited the rough coats, red eye exudate, and postural imbalance already noted. In such instances, though a single large dose of vitamin A quickly brought the visual threshold back to normal, as shown in Figure 8, the external appearance was still poor. Several days elapsed before the weight began to increase, and it took several weeks before they again looked sleek, though no more vitamin A had been administered.

Conclusions.—These experiments reveal a simple and consistent series of changes

directly related to the availability of vitamin A.   On being deprived of vitamin A, the rat exhausts its store of this substance in the liver at a regular rate, meanwhile maintaining the levels in the other tissues.   With the liver emptied, the blood level falls, and shortly afterward evidences of tissue deprivation appear.   The first of these is night blindness—the visual pigments lose their prosthetic group, vitamin A aldehyde.   Later the protein components of the visual pigments, the opsins, also decline; but this is a secondary phenomenon accompanied by general signs of tissue disintegration in the eye and elsewhere in the organism.   On administering vitamin A, all these changes are reversed.

For some years past we have had to face the embarrassment that the only function of vitamin A in the organism that we understand—that of supplying the chromophores of the visual pigments—plays only a trivial part in the whole complex of vitamin A deficiency.   No animal dies of night blindness.   Vitamin A must have some general and fundamental function in tissues all over the body, perhaps particularly in epithelia, so that in its absence the organism as a whole deteriorates and eventually succumbs.   The nature of this general function is still unknown.

It has recently become increasingly evident that the protein opsin is stabilized chemically by its combination with retinene.   Opsin, for example, is denatured much more readily than rhodopsin, by acids and alkalies[23] and by heat.[24]   We have supposed that possibly for this reason opsin can be maintained in the rods and cones only through its opportunity to form visual pigments; and that, when this opportunity is lost, as in vitamin A deficiency, the opsins also might soon be lost.   It is in part this consideration that led us to perform the present experiments.

They come out well in this regard.   It is true, whether for this reason or another, that, shortly after opsin ceases to be saturated with retinene, it begins to leave the retina; and, since opsin is an important structural constituent of the outer segments of the rods, it is perhaps for this reason that the rods deteriorate anatomically.   The important point is that these things do not happen alone but as part of a wide pattern of tissue disintegration all over the body.   The outer segments of the rods are only one among many tissues, and opsin may be only one of many tissue proteins stabilized by the presence of vitamin A or its derivatives and doomed in its absence.

We should like, therefore, to suggest that opsin may behave here as a representative protein and that the mechanism of its stabilization in the rods and cones may be representative also.   This would mean that many tissues contain structural proteins which are stabilized by direct combination with vitamin A or its derivatives and which are lost with the consequent anatomical disintegration of the tissues in the absence of vitamin A.   In this sense opsin and the rod outer segments may provide a model for the general function of vitamin A in the tissues.

[1] E. V. McCollum and N. Simmonds, *J. Biol. Chem.*, **32**, 181, 1917; C. E. Bloch, *J. Hyg.*, **19**, 283, 1920–21.   It is important to distinguish the night blindness caused by vitamin A deficiency from various types of hereditary night blindness found in man and other animals (cf. Sir W. S. Duke-Elder, *Text-book of Ophthalmology* [St. Louis: C. V. Mosby, 1944], 1, 982).   The latter seems

to correspond to a permanent congenital failure of rod function.   We know of no adequate histologic studies in man.   In certain mutant mice the layer of rods is missing, though the remainder of the retina appears normal (C. E. Keeler, *J. Exptl. Zool.*, **46**, 355, 1927).   The present observations suggest that some forms of hereditary night blindness may possibly involve the failure to synthesize the specific protein of the rods—rod opsin; the comparable hereditary disease, day blindness, might similarly be caused by the failure to synthesize cone opsin.

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